Amendments to the Specification

Please amend the specification as follows:

At the Title:

Method and Apparatus for ~~Counterconcurrent~~ Countercurrent Chromatography

At paragraph [0007]:

Some countercurrent chromatography systems utilize a complex hydrodynamic motion in two solvent phases within a column comprising a rotating coiled tube. If, for example, a horizontally mounted coil is filled with water and is rotated around its own axis, any object, either heavier or lighter than the water present in the column will tend to move toward one end of the coil. This end is then called the "head" and the other end, the "tail" of the coil. When the coil is filled with two immiscible solvent phases, the rotation establishes a hydrodynamic equilibrium between the two solvent phases, where the two phases are distributed in each turn at a given volume ratio (equilibrium volume ratio) and any excess of either phase remains at ~~either the head or~~ the tail of the coil ~~for each solvent respectively~~.

At paragraph [0008]:

When one of the solvent~~s~~ phases is added to the coil ~~from the~~ at its tail end and is further eluted from the coil ~~from~~ at its head end, the hydrodynamic equilibrium tends to maintain the original equilibrium volume ratio of the two phases in the coil and thereby a certain volume of the other phase is permanently retained in the coil while the two phases are undergoing vigorous agitation with rotation of the coil. As a result, the sample solutes present in one phase and introduced locally at the inlet of the coil are subjected to an efficient partition process between the two phases and are chromatographically separated according to their partition coefficients.

At paragraph [0024]:

Although embodiments of the invention have various applications, many advantageous embodiments of the present invention are directed to an improved ~~column~~ apparatus for use in countercurrent chromatography. Applicable chromatography techniques include those using synchronous planetary motion such as X-type, J-type, and I-type chromatography. The apparatus and methods described herein are especially advantageous when applied to high-speed countercurrent chromatography (HSCCC) with high flow rates. The column design may also be employed in large column applications for industrial-scale separations of samples by mounting the column assembly on a slowly rotating horizontal shaft. Some aspects of the invention are based, in part, on the surprising discovery that the retention of the stationary phase is improved if the configuration of the column used in ~~counter-current~~ countercurrent chromatography is modified from coiled tubing to a series of grooved plates (also referred to herein as separation disks) forming a plurality of interleaved spiral channels. The centrifugal force gradient produced by the spiral pitch in the separation disks helps to more efficiently distribute the heavier phase in the periphery and the lighter phase in the proximal portion of the column.

At paragraph [0025]:

This centrifugal force effect on a sample is enhanced by increasing the pitch of the spiral, and a spiral column assembly prepared by simply winding tubing into a flat spiral configuration like thread on a spool provides only a limited spiral pitch. In accordance with these observations, a column for use in high speed ~~counter-current~~ countercurrent chromatography has been developed having interleaving, grooved separation disks with multiple flow channels instead of coiled tubing. The use of a column having interleaving, grooved separation disks has many advantages over the prior art. First, a column possessing a plurality of interleaving, grooved separation disks with multiple flow channels provides a greater spiral pitch than previous column designs, facilitating the movement of a fluid sample radially outward at a faster rate than with previous designs, thereby providing a more efficient separation of the two phases of a fluid sample. Finally, the column design of the present invention obviates the shortcomings of earlier column designs inasmuch as it is easier to manufacture.

At paragraph [0028]:

Still with reference to **FIG. 1**, ~~a pair of flow tubes~~ an inlet flow tube **24** ~~,~~ and an outlet flow tube **26** ~~are led~~ may pass through a center ~~hole~~ bore in the column holder shaft **20** downward and out of the column assembly **10** to exit the complete centrifuge apparatus (not shown), for example at a center hole in an upper plate of the ~~whole~~ complete centrifuge ~~assembly~~ apparatus ~~where~~and the ~~flow tubes~~ inlet flow tube **24** ~~,~~ and outlet flow tube **26** may be tightly fixed with a pair of clamps (see, for example, the routing shown in U.S. Pat. No. 4,430,216). Advantageously, ~~these~~ flow tubes **24** ~~,~~and **26** may be protected with a sheath of flexible tubing such as Tygone (Norton Company, Worcester, Mass.) to prevent direct contact with metal parts in the complete centrifuge apparatus ~~assembly~~.

At paragraph [0029]:

FIG. 2 is an exploded view of the components of the column assembly illustrated in **FIG. 1**. The column assembly **10** comprises an upper flange **12** having a gear **22** and a lower flange **14** . Disposed between the upper flange **12** and the lower flange **14** ~~is~~ are a plurality of ~~coupled~~ fluidly connected separation disks **16a-16b**. Alternating between each of the plurality of separation disks **16a-16b** is a septum **18a** . Another septum **18b** is situated between the upper flange **12** and the uppermost separation disk **16a**, and yet another septum **18c** is situated between the lowermost separation disk **16b** and the lower flange **14**. The separation disks **16** can be constructed from stainless steel or a plastic such as a PTFE, high density polyethylene, or any other suitable polymer. Advantageously, the separation disk **16** may have a diameter of between 1 cm and 30 cm, and a thickness of between 0.5 and 20 mm. In one embodiment that has been found suitable, the separation disk **16** has a diameter of about 17.5 cm and a thickness of about 4 mm. Preferably, the septum **18** is constructed of PTFE (e.g. Teflon®, E.I. Du Pont, Wilmington, Del.). One of skill in the art would appreciate that the septum **18** can be constructed from any number of suitable non-reactive materials. The construction of the separation disks **16** and septa **18** are described in additional detail below with reference to **FIGS. 3, 4A, and 4B**.

At paragraph [0030]:

Turning now to **FIG. 3**, a first embodiment of a separation disk **16** having a first surface **30** and a second opposed surface **32** is illustrated. The separation disk **16** includes an inner edge **40** and an outer edge **42**. The separation disk **16** comprises a single spiral flow channel **44** carved, etched, or molded on the surface of the first side **30** of the separation disk **16**. The spiral flow channel **44** has an inlet end **46** and an outlet end **48** with fluid flow typically traveling along the path of the spiral channel **44** from the inlet end **46** to the outlet end **48**. Advantageously, the spiral channel **44** of one separation disk **16** is serially connected to the spiral channel **44a** of another separation disk **16a** (not shown) by stacking multiple separation disks **16** adjacent to one another with ~~a septum~~ septa **18** separating each pair. Preferably, the outlet end **48** of the channel **44** connects to the inlet end **46a** of the channel **44a** on the next adjacent disk **16a** (not shown). To accomplish this, the bottom of the outlet end **48** of the channel **44** includes ~~a hole~~ an outer hole **62** (which may be about 1 mm diameter) that is connected to a radial channel **49** grooved or molded into the ~~other side~~ second opposed surface **32** of the separation disk **16**, ~~with a hole (which may be about 1 mm diameter,)~~ that extends through the thickness of the separation disk **16**. The radial channel **49** ~~on~~ grooved within the ~~other side of the disk~~ second opposed surface **32** extends radially inward and ~~until it is~~ substantially ~~aligned~~ aligns with the inlet end **46** of the spiral channel **44** and is physically separated from the inlet end **46** by the material of the separation disk **16**. An inner~~-end~~ hole **64** ~~of~~ in the radial channel **49** is adjacent to a hole in the septum **18** (not shown) that connects to the inlet end **46a** of the spiral channel **44a** on the next adjacent disk **16a** (not shown).

At paragraph [0031]:

To hold the separation disks **16** together in making the whole column assembly **10**, ~~the~~each separation disk **16** advantageously includes a plurality of screw holes **50** at regular intervals ~~at~~ near both the inner **40** and outer **42** edges of the separation disk **16**. In some suitable embodiments, the screw holes **50** are positioned circumferentially at approximately 10 degree~~s~~ spacings for the outer edge **42** and 45 degree~~s~~ spacings for

the inner edge **40**. Similar holes (not shown) are also made in both the septa **18** and the flanges **12 and 14** as will be described in greater detail with reference to **FIGS. 5, 6, and 7**.

At paragraph [0032]:

In a preferred embodiment, multiple interleaved spiral flow channels are incorporated symmetrically around the center of a separation disk **16** so that the spiral pitch is increased as compared to the spiral pitch of a single spiral channel **44** such as is shown in **FIG. 3**. **FIG. 4A** is a top view of the first surface **30** of a separation disk **16** having a plurality of interleaved spiral channels **52, 54, 56, and 58**. **FIG. 4B** is a cross section of the separation disk **16** of **FIG. 4A**, and **FIG. 4C** is a top plan view of the separation disk **16** of **FIGS. 4A** and **4B**. As illustrated in these Figures, the separation disk **16** has four interconnected ~~separate~~ spiral channels **52**, **54**, **56**, and **58** ~~, respectively~~. However, it will be appreciated that the number of spiral channels can vary. Each channel may be between 0.25 mm and 10 mm wide. Preferably, the width of each channel may be between 0.5 mm and 7 mm wide, with 3 mm having been found suitable in one embodiment. The depth of each channel can likewise vary. Preferably, the depth of each channel is between 0.1 mm and 5 mm, with 2 mm having been found suitable in one embodiment. Depending on the size of the disk, each channel may have a length of between about 250 mm and 5 m. In one advantageous embodiment, the length of each channel is approximately 1 m. It will be appreciated, however, that the length of each channel can vary. Each groove or channel is separated from the next groove or channel by a ridge **60**, which may measure approximately 1 mm in width for 3 mm wide channels.

At paragraph [0033]:

Still with reference to **FIG. 4A**, each channel **52, 54, 56, and 58** has an inner end denoted **I1**, **I2**, **I3**, and **I4** respectively in **FIG. 4A**. The channels **52, 54, 56, and 58** each begin at their inner ends **I1, I2, I3, and I4** and spiral around to their outer ends denoted **O1**, **O2**, **O3**, and **O4** respectively in **FIG. 4A**. In the embodiment of ~~FIG. 3B~~ **FIG. 4A**, each channel **52, 54, 56, and 58** forms ~~3.25~~ 2.75 spiral turns so that the outer end of a

given channel is at the same angular location relative to the inner end of the next channel. Thus, as shown in **FIG. 4A**, **O1** is at the same angular orientation as **I2**, **O2** is at the same angular orientation as ~~I 2~~ **I3**,~~ etc.~~ and **O3** is at the same angular orientation as **I4**.

At paragraph [0034]:

Connection between ~~an~~ the outer ~~outlet~~ end of one spiral channel **O1**, **O2**, and **O3** and ~~an~~ the ~~inlet~~ inner end **I2**, **I3**, and **I4**, respectively of the next spiral channel is made by ~~another~~ connecting channels **72**, **76**, and **78**, respectively, that are formed on the second opposed surface **32** of the separation disk **16** as illustrated with dashed lines on **FIG. 4A** and also on **FIG. 4~~B~~C**, which shows a top plan view of the second opposed surface **32**. Except for the ~~inlet~~ inner end **I1** of the first channel **52** ~~(I1)~~ and the outer end **04** of the last channel **58**, each end of the channels **52, 54, 56, and 58**, have a hole **70** (approximately 1 mm in diameter) through the separation disk **16** which communicates with the end of ~~a~~ the connecting channels **72**, **76**, or **78** on the ~~opposite~~ opposed surface **32** of the separation disk **16** .

At paragraph [0035]:

Referring now to FIGS. **4A**, **4B**, **4C**, and **5**, when a sample solution is introduced into the column assembly **10**, fluid enters the inner end **I1** of the first channel **52** and travels in the direction of the spiral to the outer end **O1** of the first channel **52**. At this point, the fluid travels through hole **70** to the end of the ~~linear~~ connecting channel **72** on the~~ other side of the disk~~ opposed surface **32** of the separation disk **16**, thereby providing a flow path for the sample to travel from the outer end **O1** of the first channel **52** to the radial location of the inner end **I2** of the next channel **54**. From there the fluid flows through another hole **70** ~~extending between the inner end of the linear~~ connecting channel **72** and the inner end **I2** of the second spiral channel **54**. The fluid then travels around to the outer end **O2** of the second channel **54**, where it passes through a hole **70** to the connecting ~~radially extending~~ channel **78** on the ~~other side of the disk~~ opposed surface **32** of the separation disk **16**, ~~from~~ where it travels radially to the location of the inner end **I3** of the third channel **56**. This process is repeated through the third ~~and fourth~~

~~channels~~ channel **56**, connecting channel **78**, and fourth channel **58** until the fluid ~~ends up~~ emerges at the outer end **O4** of the fourth channel **58**. At this point, the fluid passes through another hole **70** at the outer end **O4** of the fourth channel **58** and through ~~radial~~ connecting channel **80** to the radial position of inner end **I1** where the flow started. However, no hole through the separation disk **16** to the inner end **I1** of the first channel **52** is provided. Rather, an interdisk connection hole **84** in the septum **18** is positioned to coincide with the location of the inner end **I1** of the ~~flow~~ first channel ~~**80**~~ **52** on one separation disk **16** and with the inner end **I1a** of the first channel **52a** on the next adjacent separation disk **16a** (not shown). The interdisk connection hole **84** conducts the fluid from the connecting channel **80** of the separation disk **16** to the inner end **I1a** of the next adjacent separation disk **16a** (not shown). Thus, in a series of adjacent separation disks **16a-16b** as shown in **FIG. 1**, fluid passes from the outer end **O4** of the fourth channel **58** on one separation disk **16a** to the ~~beginning~~ inner end **I1a** of the first channel **52a** on the next separation disk **16b** (not shown).

At paragraph [0036]:
In operation, the pitch of each additional spiral on a separation disk **16** may increase~~s~~ markedly as compared to the pitch of a single spiral channel, assuming that the volume of fluid contained in the single spiral channel is essentially the same as the volume of fluid contained in the multiple spirals. For example, when a separation disk **16** with a diameter of about 17.5 cm includes four spiral channels, the pitch ~~can~~ of each spiral may become as large as 16 mm (three times that of the single spiral channel).

At paragraph [0037]:
One embodiment of the flanges which are placed on the top and bottom of the stack of separation disks **16** of **FIG. 1** is shown in **FIGS. 6** and **7**. The upper flange **12** (**FIG. 6**) is equipped with a gear **22** which engages with a stationary gear on the HSCCC centrifuge (not shown). **FIG. 7** depicts the lower flange **14** which has two screw holes **90 , 92** positioned substantially 90 degrees apart circumferentially for tightly fixing the column assembly **10** against the column holder shaft (0.9 inch diameter). Both the upper and lower flanges **12 , 14** each have an inlet/outlet hole **94** which fits to an adapter (not

shown) with a screw thread. They also have a set of screw holes ~~**60**~~ **66** located at regular intervals around the circumference near the outer and inner edges of each septum **18** ~~as in~~ in alignment with the screw holes **50** of the separation disks **16** and ~~Teflon~~ septa **18**. In preferred embodiments, the screw holes ~~**60**~~ **66** are positioned circumferentially at approximately 10 degree~~s~~ spacings ~~apart~~ for the outer edge and 45 degree~~s~~ spacings ~~apart~~ for the inner edge.

At paragraph [0038]:
It has been observed that the use of a rectangular spiral channel embedded in a solid separation disk **16** as described above enhances the retention of the stationary phase for viscous, low interfacial tension two-phase solvent systems. Accordingly, in one embodiment, a column assembly **10** having a separation disk **16** comprising at least one rectangular spiral channel **44** embedded in the separation disk **16** is provided. The rectangular spiral channel configuration has a number of advantages over the prior art. For example, the rectangular spiral channel is useful for separating biopolymers such as proteins, DNA, RNA, polysaccharides, and cell particles. Additionally, this channel design ensures reliable retention of the stationary phase for polar or low interfacial tension solvent systems such as the 1-butanol/water system to separate bioactive compounds including peptides. Similarly, the rectangular design provides an improved stationary phase retention against emulsification.

Amendments to the Drawings

Please replace the drawings for Figures 1, 2, 3, 4A, 4B, 4C and 6 with replacement sheets for these drawings submitted herewith.